A Modified Technique for Mounting Thysanoptera in Canada Balsam [1]

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Abstract

A technique is described for mounting Thysanoptera in Canada Balsam which overcomes most of the problems associated with the use of this mounting medium. It is unnecessary to puncture the animals prior to clearing them and usually the appendages are already spread. In addition the male external genitalia are extruded.

Introduction

Attempts have been made to shorten the process of mounting Thysanoptera on slides by using mounting media such as glycerine, glycerine-gelatin, Berlese's , Hoyer's, de Faure's, polyvinyl lactophenol etc. (Priesner, 1960). An important advantage that all of these media share is that the animals can be mounted into them directly, either alive or from alcohol. In addition, some of them (e.g., Hoyer's, Berlese's) are water-based and facilitate study of the animals by phase microscopy (Ross in Stannard, 1968). However, none of these preparations are permanent even if they are ringed. Also, the specimens tend to fade with time and frequently the taxonomically-important ocellar and epidermal pigments of the thrips dissolve (Priesner, 1960). For these reasons most thysanopterists agree that the best mounting medium for thrips is Canada Balsam (Hartwig, 1952; Bierne, 1955; Priesner, 1960; Faure, 1961; O'Neill and Bigelow, 1964; Stannard, 1968).

Unfortunately, the use of Canada Balsam has three major drawbacks. First, considerable time and patience is required to process the specimens properly prior to mounting them. Second, the specimens tend to crumple or shrink when they are transferred from 95%, 98%, or absolute alcohol to the usual clearing agents (xylol, carboxylol, oil of wintergreen, oil of cloves, etc.). To avoid this problem the usual procedure is to puncture the abdomen prior to transferring each specimen to the clearing agent (Hartwig, 1952; Priesner, 1960; Stannard, 1968). Unless the worker is experienced, this process often results in the damage or removal of structures (sclerites, setae) necessary for the identification of the thrips. Third,

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the appendages (wings, legs), of the specimen must be spread before the coverslip is applied. This operation is often unsuccessful and, again, frequently results in the destruction of useful characters.

Having recently begun a comparative morphological study of the male external genitalia of thrips, I have experimented with various mounting methods to find one the use of which would result in the preparation of specimens with everted genitalia. This search has resulted in the development of a technique which avoids some of the drawbacks of Canada

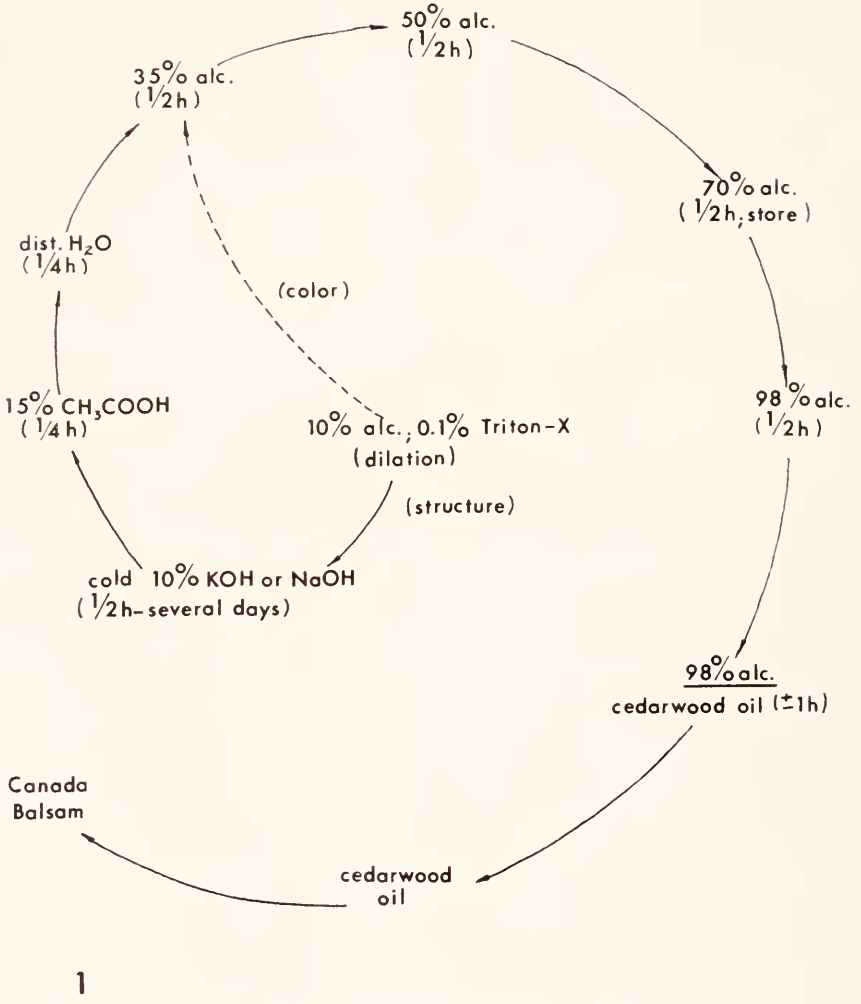

Fig. 1. Outline of mounting procedure.

Balsam noted above. The technique is basically that of Hartwig (1952) as modified by Faure (1958).

Procedure

The mounting procedure is outlined in Fig. 1. Thrips are collected in the usual manner (Bailey, 1957; Priesner, 1960) into 10% ethanol (Hartwig, 1952) to which 0.1% Triton-X emulsifier has been added (Faure, 1958). The low alcohol concentration, over a period of two days to a week or more, distends the thrips, extends the wings (in Terebrantia; in Tubulifera the wings are often held over the abdomen by wing-holding setae) and legs and everts the male and female external genitalia (this also occurs in small Coleoptera, Hymenoptera, Diptera, etc. collected with the thrips). As Hartwig (1952) pointed out, distension is facilitated at higher temperatures (37° C) and by placing grass seeds (preferably germinating) in the collecting solution. The emulsifier acts as a wetting agent and causes the thrips to sink.

Further processing is best carried out in 70 mm watch glasses, each collection of thrips remaining in the glass as the succession of liquids are introduced and drawn off with a medicine dropper. Between changes a second, 100 mm, watch glass inverted over the first keeps out dust. The collecting label for each collection is placed under the watch glass. In this way one can process up to 15 collections, each containing many individuals, one after the other, thereby eliminating the time factor as a drawback in the use of Canada Balsam.

The thrips are passed successively through 35%, 50%, and 70% ethanol, leaving them in each concentration for at least ½ hour. The thrips can be stored in 70% ethanol, but over long periods (several months or more) the epidermal and ocellar pigments and later, the integumentary ones, fade (Priesner, 1960).

If the thrips are dark and heavily sclerotized (e.g., *Elaphrothrips, Haplothrips*, etc.) a few of the series should be macerated in cold 10% KOH or NaOH for from ½ hour to several days, depending on degree of sclerotization. This treatment reveals many structures, necessary for identification, which would be invisible otherwise. Others of the series should not be macerated because of the importance of color. After maceration, the lye is replaced by 15% acetic acid followed by distilled water, both for 15 minutes. Following this the specimens are treated in the usual manner. (The amount of time the specimens were in lye should be indicated on the label after mounting, Stannard, pers. comm.).

The thrips are placed in 98% or absolute alcohol for at least ½ hour before infiltrating them with cedarwood oil. Cedarwod oil is used because

it is much heavier than the alcohol. An embryological staining dish is half-filled with the oil. If the dish is then *slowly* filled to the top with absolute ethanol, the two liquids will remain separate for several hours, the lighter alcohol floating on the cedarwood oil. Each thrips is lifted out of the watch glass with a camel's hair brush and an insect pin is used to transfer it from the brush into the staining dish. The thrips sink through the alcohol and come to rest on the surface of the cedarwood oil. Over the next hour or so, as the thrips become infiltrated, they gradually sink through the cedarwood oil and, when completely clear, some to rest on the bottom of the dish. No shrinkage occurs and the male genitalia remain expanded. The entire collection is then transferred in a medicine dropper to a syracuse watch glass filled with pure cedarwood oil. Mounting, labelling and drying are carried out in the usual fashion (Hartwig, 1952; Bierne, 1955; Priesner, 1960; O'Neill and Bigelow, 1964; Stannard, 1968, pers. comm.), but it is usually unnecessary to spread the appendages except to flip the wings of phlaeothripids out from under the wing-holding setae. When the alcohol in the embryology dish starts to become milky (usually after three to four hours) the infiltration solution should be thrown out and a new one prepared.

Observations and Discussion

Figs. 2 and 3 are photomicrographs of a brachypterous male *Chirothrips patruelis* Hood and a macropterous female *Anaphothrips cameroni* (Bagnall) mounted according to the procedure outlined in this paper. Note that the antennae, legs and wings are nicely spread as in mounts prepared in the traditional manner by experienced thysanopterists. Since all speci-

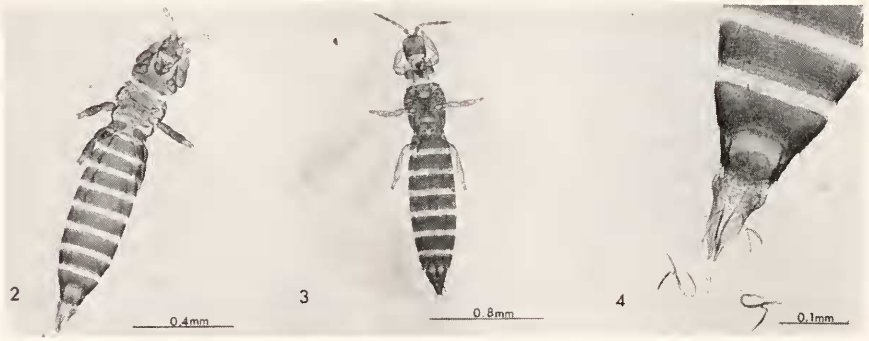

Fig. 2. *Chirothrips patruelis* Hood, brachypterous male, dorsal aspect. Fig. 3. *Anaphothrips cameroni* (Bagnall), macropterous female, dorsal aspect. Fig. 4. *Chirothrips patruelis* Hood, extended male genitalia, dorsal aspect.

mens mounted in this way are distended, measurements for taxonomic purposes should be taken as outlined by Priesner (1960).

Fig. 4 is a photomicrograph, taken at higher magnification, of the distended male genitalia. Both Hartwig (1952) and Priesner (1960) advise separating the genitalia from the animal and mounting them separately under another cover slip on the same slide. If very little Canada Balsam is used, the genitalia flatten on drying, and the preparation is easier to study. The external genitalia are being used with increasing frequency in taxonomic studies of thrips, particularly the aedeagus or pseudovirga of the genitalia of male Phlaeothripidae (see recent papers of Priesner, Faure and zur Strassen). From my own work, I predict that the shape and ornamentation of the epiphallus or endotheca in Terebrantia will be of use in higher taxon evaluation (the terminology of the various parts of the genitalia is discussed by Hartwig, 1952; Priesner, 1956, 1960; and Heming, 1970).

This technique has two minor disadvantages. When female phlaeothripids are mounted in this way the terminal portions of the reproductive system are usually everted, bringing the spermatheca and aciculae and frequently the fustis outside of the animals. This makes comparison of these structures between specimens difficult.

The other problem is associated with the ovipositor of female Terebrantia. This structure is usually extended at right angles to the body when the animal is processed as outlined above. When the cover slip is placed on a specimen mounted with the dorsum uppermost, the ovipositor tends to be bent sideways, twisting the abdomen to one side or the other. However this makes lateral mounts of female terebrantians, necessary for studying ovipositor characters, easier to prepare.

The technique outlined in this paper should be useful for studying genitalia of other small insects, particularly those of small beetles.

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The Buffalo Treehopper on a Mississippi River Island (Homoptera, Membracidae).—In a previous paper (1969, Ent. News 80: 32–33) I had described the incidences of treehoppers on islands in the Mississippi River between miles 621 and 623 on the Corps of Engineers navigation chart.

During late summer and autumn of the years 1967, 1968 and 1969 the buffalo treehopper, *Stictocephala bubalus* (Fabricius), was collected from an island at mile 619. This island is the southerly one of the pair just east of the 619 mile designation on the chart.

Goldenrod is growing on this island and hosts the membracid. Neither the buffalo treehopper nor this plant have been found on the islands between miles 621 and 623. Other plants from which this insect has been collected such as American elm, haw, nettle and wild plum grow on the latter islands but have failed to produce the hopper. There is an abundant growth of American elm; but haw, nettle and wild plum are uncommon. These four plants are not favored hosts of the insect in mainland areas; here alfalfa, apple, clovers and goldenrod are preferred. Apparently, when one of the preferred hosts is found, so is the insect.

It is a matter of conjecture how the islands were colonized by membracids in the first place. Perhaps some were left on the islands formed by rising water after the dams were built several years ago. Winds may have been responsible. Possibly they could have flown onto the islands. They are not good distance fliers and often when disturbed fly out a short way and return to near the point they had left. Still, they could have flown the short distance required in some places. And these insects could have arrived as eggs, nymphs or adults with vegetative debris during the flooding periods.

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